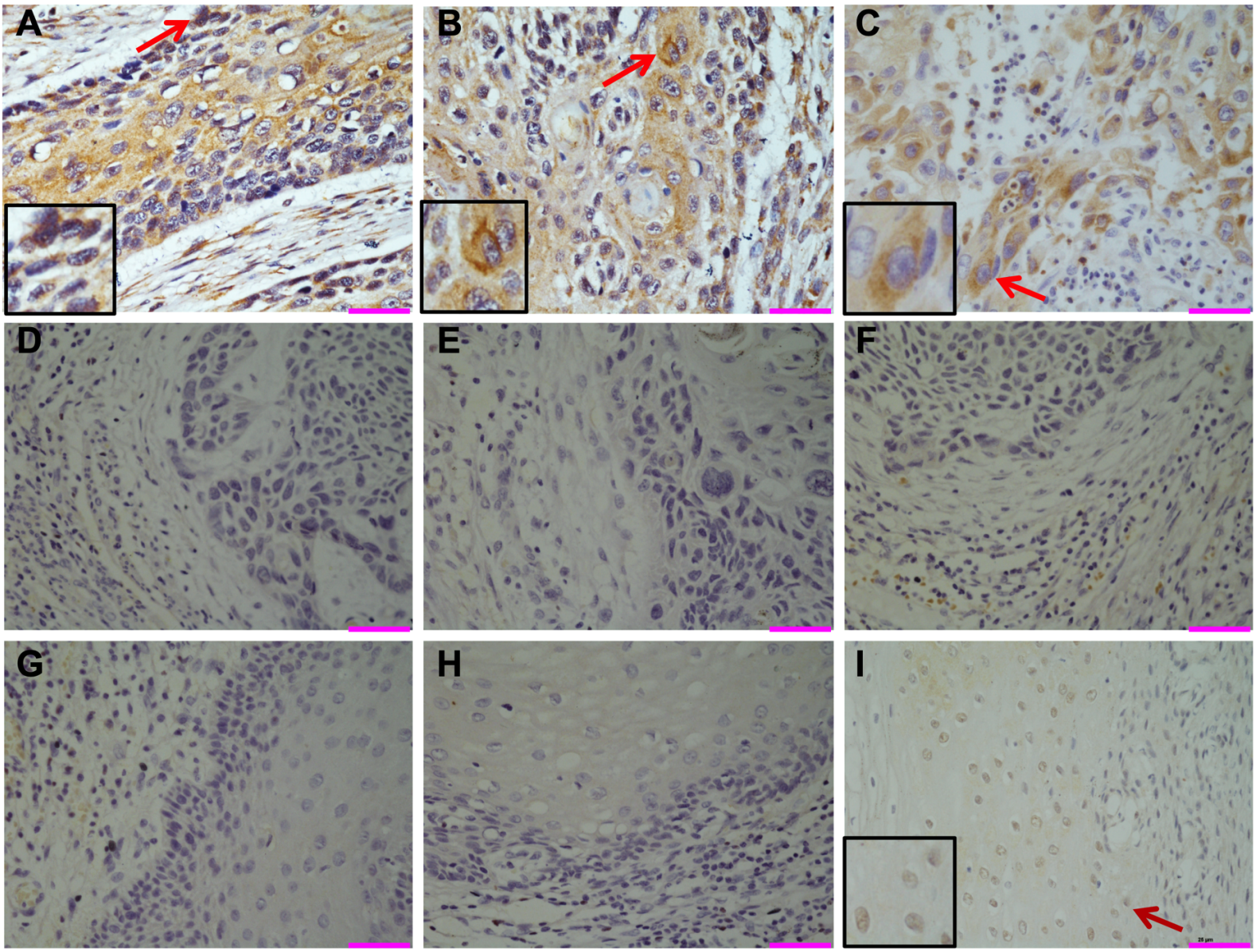
Supplemental Table S1

Table S1. Clinicopathologic factors associated with the infection of *P. gingivalis* in patients with ESCC before and after PSM

	Before Matching				After Matching				
	Pg (+) (n=141)	Pg (-) (n=171)			Pg (+) (n=113)	Pg (-) (n=113)			
Variable			95%CL	P Value			95%CL		P Value
Gender									
Female	36(25.5%)	71(41.5%)	0 to 0.016	0.004	29(5.7%)	35(31.0%)	0.431	to	0.46
Male	105(74.5%)	100(58.5%)			84(74.3%)	78(69.0%)	0.529		
Age									
< 60	50(35.5%)	44(25.7%)	0.019 to 0.056	0.082	42(37.2%)	38(33.6%)	0.639	to	0.676
≥60	91(64.5%)	127(74.3%)			71(62.8%)	75(66.4%)	0.731		
Smoke									
Yes	85(60.3%)	56(32.7%)	0 to 0.007	<0.001	65(57.5%)	57(50.4%)	0.303	to	0.35
No	124(66.3%)	115(67.3%)			48(42.5%)	56(49.6%)	0.397		
Drink									
Yes	69(48.9%)	39(22.8%)	0 to 0.007	<0.001	55(48.7%)	44(38.9%)	0.145	to	0.18
No	72(51.1%)	139(74.3%)			58(51.3%)	69(61.1%)	0.22		
Differentiation									
I	34(24.1%)	32(18.7%)	0.277 to 0.368	<0.490	28(24.8%)	20(17.7%)	0.431	to	0.238
II	69(48.9%)	92(53.8%)			55(48.7%)	61(54.0%)	0.529		
III	38(27.0%)	47(27.5%)			30(26.5%)	32(28.3%)			
Invasion depth									
< muscularis	36(25.5%)	80(46.8%)	0 to 0.007	<0.001	35(31.0%)	42(37.2%)	0.357	to	0.4
≥muscularis	105(74.5%)	91(53.2%)			78(69.0%)	71(62.8%)	0.453		
Lymphatic metastasis									
Yes	92(65.2%)	43(25.1%)	0 to 0.007	<0.001	64(56.6%)	50(44.2%)	0.06	to	0.084
No	49(34.8%)	128(74.9%)			49(43.4%)	63(55.8%)	0.115		
TNM									
I&II	61(43.3%)	139(81.3%)	0 to 0.007	<0.001	61(54.0%)	75(66.4%)	0.053	to	0.077
III&IV	80(56.7%)	32(18.7%)			52(46.0%)	38(33.6%)	0.107		

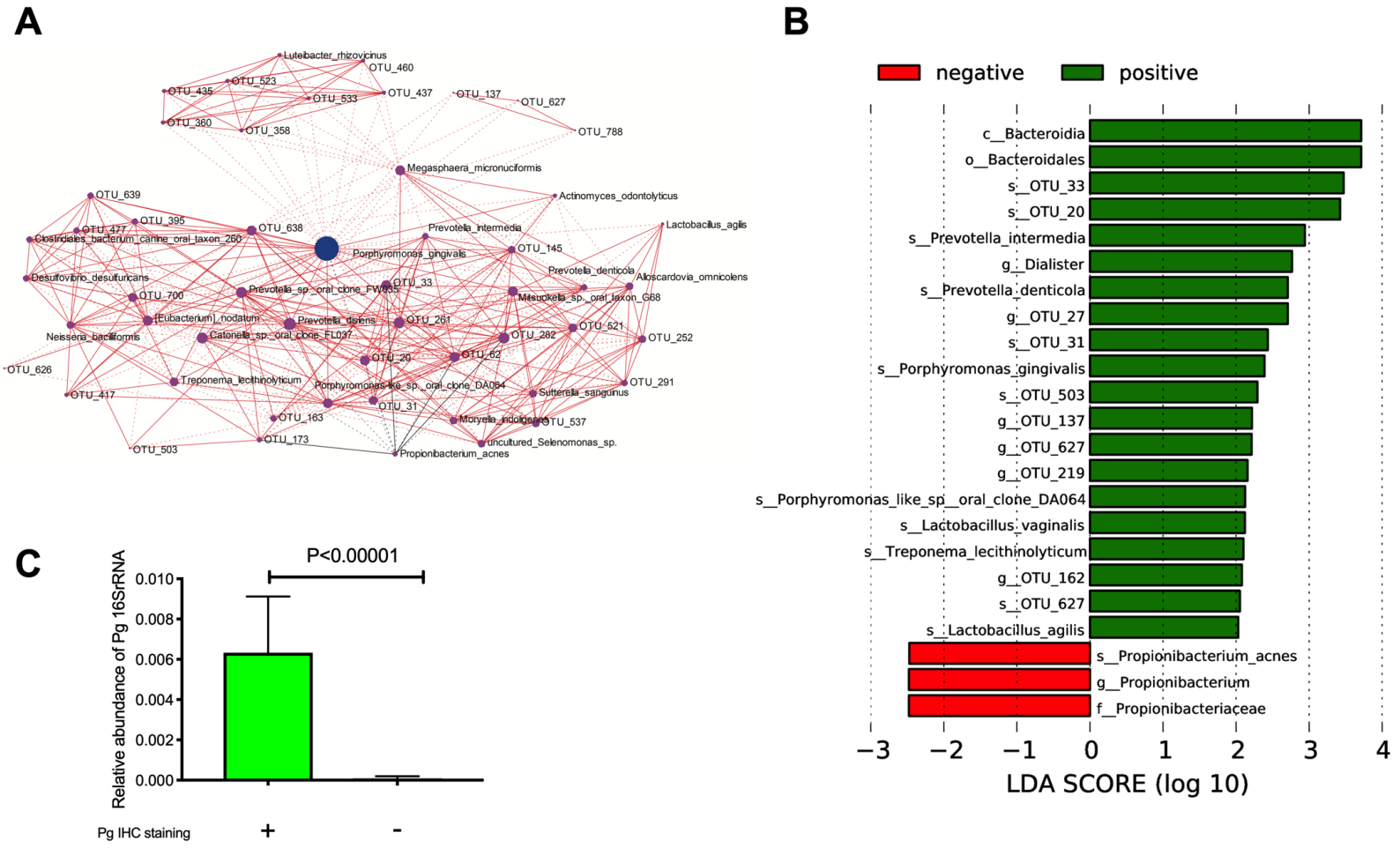
Supplemental Figure S1



Immunohistochemical detection of *P. gingivalis* in normal esophageal mucosa, and cancerous and adjacent tissues of ESCC.

A, **B**, and **C** are representative images of *P. gingivalis* antigen present in well differentiated (**A**), moderately differentiated (**B**), and poorly differentiated (**C**) ESCC tissues. Pre-immune rabbit IgG was used as a control in serial tissue sections from the same paraffin-embedded tissue block of (**D**) well differentiated-; (**E**) moderately differentiated-; and (**F**) poor differentiated ESCC. (**G**) Normal esophageal mucosa stained with *P. gingivalis* antiserum; (**H**) and (**I**) are representative negative/positive images of *P. gingivalis* immunostaining in adjacent cancerous tissues. 20× magnification; scale bar = 50 μm; miniatures in the left corners amplify the area with *red arrows*.

Supplemental Figure S2



Infection with *P. gingivalis* alters the composition of esophageal microbiome Nested PCR amplification-based 16S rRNA sequencing of the V4-V5 regions was performed to examine the possible influence of *P. gingivalis* on the composition of the microbial community in cancerous tissue samples (n=50) of ESCC patients. (A) Bacterial abundance in esophageal cancerous tissues is associated with the infection of *P. gingivalis* shown in a network (solid line represents positive association with *P. gingivalis*, while the dotted line represents a negative association). Detailed information of the association among different bacterial species is shown in S4-S5. (B) LEfSe analyses showing the distinct alteration of other esophageal bacteria by the presence of *P. gingivalis* ("g", "s", "o", and "c" represent genus, species, order and class respectively, and OTU number was used when the bacteria classifications are undetermined). (C) The relative abundance of *P. gingivalis* 16S rRNA in cancerous tissue with immunohistochemical positive staining for *P. gingivalis* antigens is significantly higher than that of negatively staining tissue.

Supplemental Methods

Cell apoptosis, viability and proliferation assays

Cells were collected and stained with fluorescence-conjugated Annexin V and PI. After washing twice with 2 ml of fluorescence-activated cell sorting (FACS) buffer, cells were fixed with formaldehyde at a final concentration of 4% in PBS for 10 min at room temperature. Cells were washed twice in PBS containing 2% FBS and analyzed immediately by flow cytometry (FACSCelesta, Becton-Dickinson, NY).

Immunohistochemistry and *P. gingivalis* staining

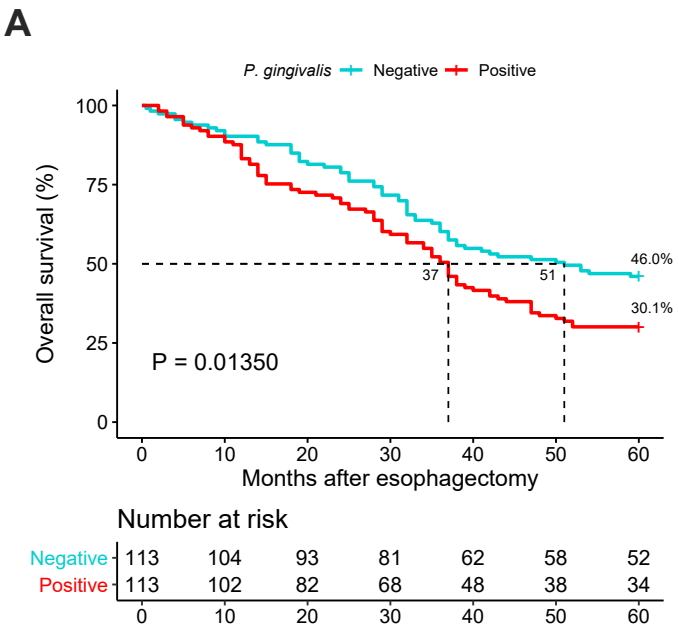
For measurement of *P. gingivalis* invasion, 0.05×10^6 KYSE-30 cells were seeded in 12 well cell culture plates and incubated at 37°C for 24 h, prior to addition of *P. gingivalis* 33277 or the isogenic Δ*fimA* mutant (MOI 10). After 6 h incubation with *P. gingivalis*, culture medium was removed and cell were washed with PBS and fixed in 4% formal dehydrate, then permeabilized with 0.5% Triton X-100. Cells were blocked with 10% normal goat serum for 1 h and overnight incubated with rabbit anti-Pg33277 antibody (1:1000), then washed with PBS and incubated with goat anti-rabbit Alexa Fluora 594 for 1 h, followed by the staining with Actin-Green 488 ReadyProbes reagent (Invitrogen, Carlsbad, CA) for 30 minutes. DNA Damage and TUNEL Assay Kit (Millipore, St. Louis, USA) was employed, according to the manufacturer's procedures, to examine cell apoptosis in the biopsies and xenograft tissues. After the completion of annexin V staining, coverslips were mounted on glass slides using ProLong Gold antifade reagent with DAPI (4′6-diamidino-2-phenylindole) mounting medium (Invitrogen, Carlsbad, CA) prior to imaging with a confocal scanning laser microscopy (Leica TCS SP8, Germany) with a 63× oil immersion objective. From a minimum of five serial tissue sections of each sample, the mean of positive staining cell (apoptotic cell) numbers, at least 20 views of each, was calculated. For the biopsy

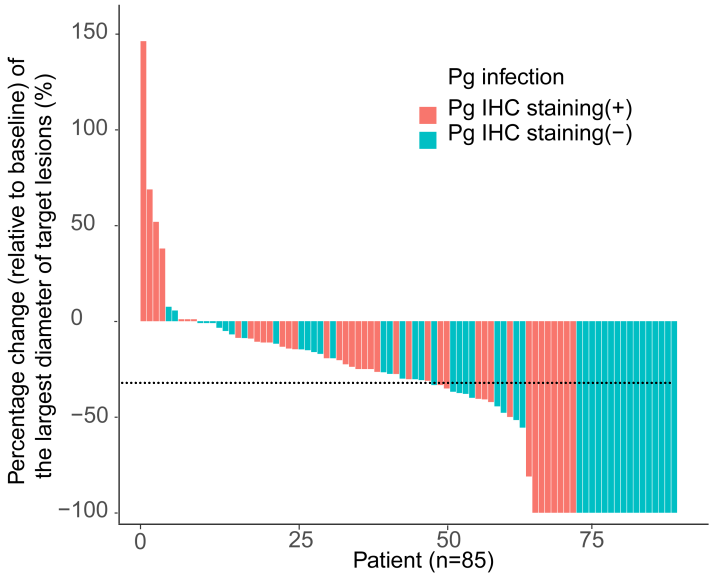
samples from 85 patients, the change of apoptotic cell numbers before and after neoadjuvant chemotherapy for each patient was used to assess their responses. Images were analyzed using Imaris Cell Imaging software (South Windsor, CT, USA).

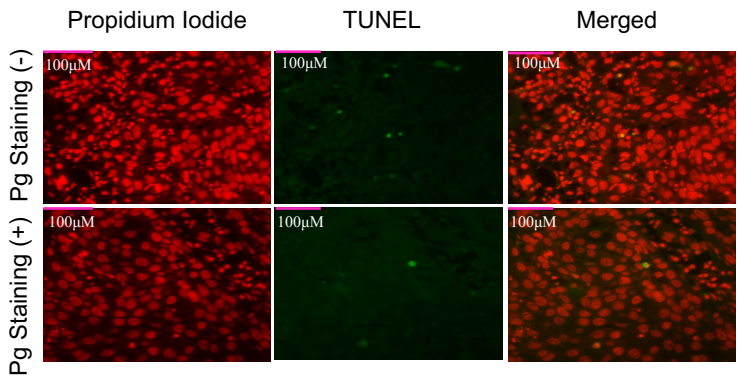
Statistical analysis

Propensity score matching was conducted, using the greedy nearest neighbor algorithm with a caliper width of 0.14 standard deviations of the propensity score (S3). An absolute standardized difference of less than 0.1 after matching was considered to indicate well-balanced matching variables between the groups. Propensity score matching yielded 113 pairs of cases, which were compared using a log-rank test with the hazard ratio (HR) derived using univariate Cox regression.

Supplemental Figure S3







D

The tumor stages of 85 ESCC patients to neoadjuvant chemotherapy

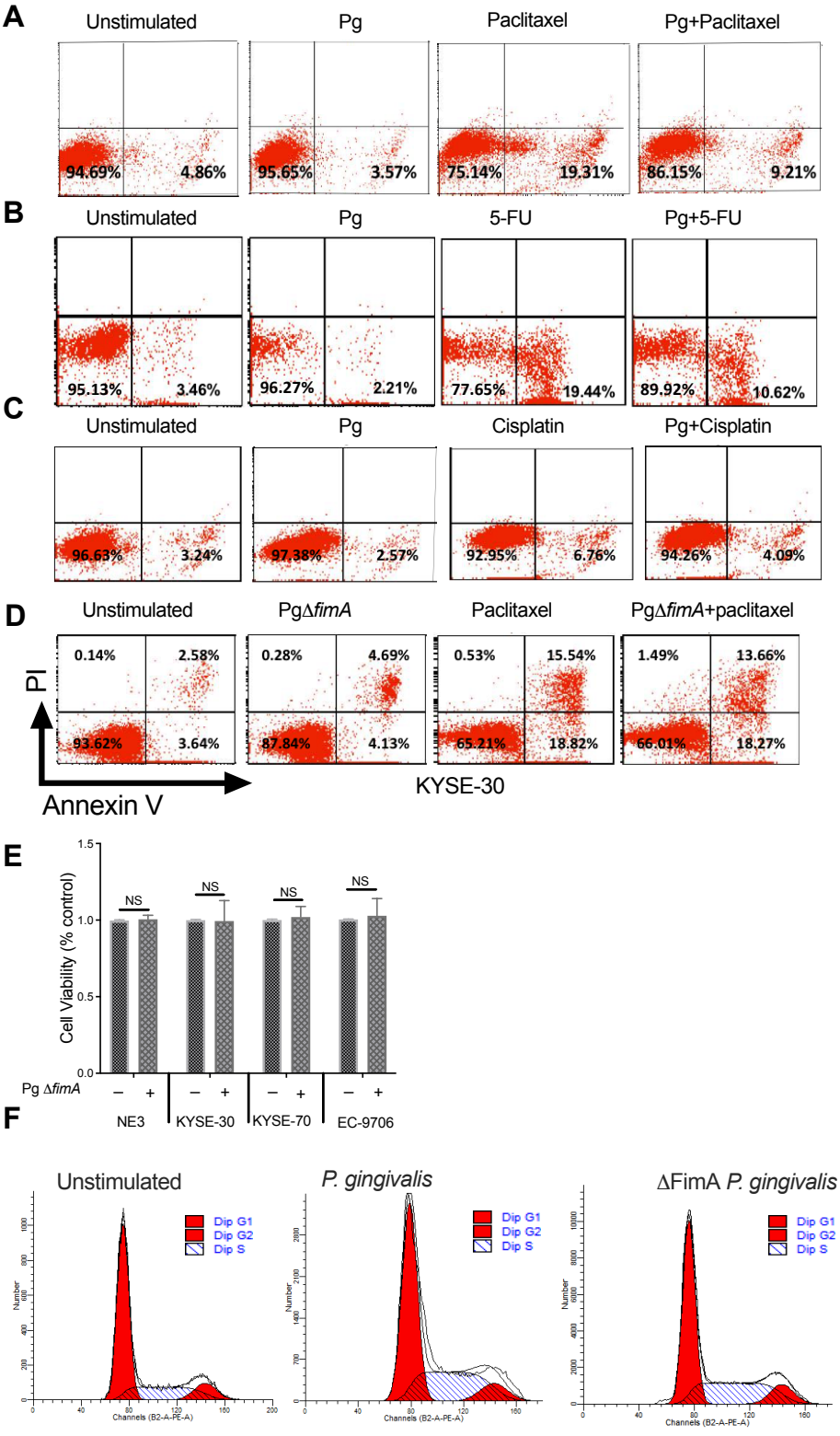
TNM stages	*P. gingivalis*		χ^2	*P* value
	Positive(n=40)	Negative(n=45)		
0(n=2)	1(2.5%)	1(2.2%)		
I(n=9)	6(15.0%)	3(6.7%)		
II(n=25)	10(25.0%)	15(33.3%)	2.238	0.692
III(n=41)	20(50.0%)	21(46.7%)		
IV(n=8)	3(7.5%)	5(11.1%)		

Data were analyzed by Chi-Square test.

Overall survival data for patients with propensity score matching and waterfall plot for the changes of the largest diameters of cancer lesions

(A) Kaplan-Meier survival analysis for 113 pairs of ESCC patients after propensity score matching, showing that *P. gingivalis* infection is negatively associated with the overall survival time of ESCC patients (*P*=0.0135). (B) Waterfall plot for the percentage changes of the largest diameters of cancer lesions for 85 patients after neuadjuvant chemotherapy (relative to those of cancer lesions before the neuadjuvant, based on CT-scanning examination). The dotted line indicates a 30% reduction of LD in target lesions. (C) Representative fluorescence images of stained apoptotic cells in cancerous tissue sections from ESCC patients with *P. gingivalis* positive- or negative-staining. Propidium iodide (red) and fluorescein isothiocyanate (FITC) (green) were used for nuclear and annexin V staining, respectively. **(D)** The American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) **TNM** (tumor, node, metastasis) classification was used to evaluate the tumor stages of the patients with ESCC. There was no significant difference observed between the groups of *P. gingivalis* infection positive and negative ESCC patients.

Supplemental Figure S4



The effects of *P. gingivalis* infection on apoptosis and proliferation of ESCC cells

KYSE-30 cells were stimulated with *P. gingivalis* 33277 or Δ*fimA* mutant (MOI 10) for 24 h followed by treatment with chemotherapy drugs (A-D) for an additional 24 h. Annexin V and PI staining were used to determine apoptotic or necrotic cell death. (A-D) Typical fluorescence images of flow cytometry of KYSE-30 cells upon treatment with paclitaxel (A), 5-FU (B), or cisplatin (C), show *P. gingivalis* infection leads to the apoptotic resistance of ESCC cells to chemotherapeutic drugs but Δ*fimA* mutant *P. gingivalis* lacks this capability (D). (E) MTT assay for viability of ESCC cells (KYSE-30, KYSE-70, KYSE-140, and KYSE-150) after challenge with the *P. gingivalis fimA* mutant for 24 h. (F) Representative flow cytometry images showing the influence of *P. gingivalis* or *fimA* mutant on the cell cycle distribution of KYSE-30, data were analyzed with ModFit software. "NS" represents not significant.